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HEALTH HAZARDS FROM HUMAN WASTE IN WILDERNESS AREAS ADMINISTERED BY THE U.S. FOREST SERVICE

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FINAL REPORT

HEALTH HAZARDS FROM HUMAN WASTE IN WILDERNESS AREAS ADMINISTERED BY THE U.S. FOREST SERVICE.

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ABSTRACT

Human feces was inoculated with Salmonella typhimurium and with a strain of Escherichia coli which was resistant to two antibiotics. Neither organism was present before inoculation. The inoculated feces was sandwiched between two soil layers in a plastic cup which had been pierced to allow water movement. Replicate cups were buried at six field sites on one mountain selected for differences in elevation, exposure and vegetation. Samples were retrieved weekly and the numbers of bacteria ascertained by plate counts. The experiment was conducted in two successive summers for eight weeks each summer.

Bacterial survival patterns for the two summers differed somewhat but in each year the survival at the end of eight weeks was on the order of 10^4 or more of each bacterium per gram of feces. The variation with site was minor in comparison to the overall survival pattern. At each site, bacterial counts of samples buried at 5 cm were identical to those buried at 20 cm. Soil temperature and soil moisture did not demonstrate any clear cut effects. Results from both years demonstrate that enteric bacteria do not disappear from buried feces as rapidly as hoped. This study should be distinguished from earlier reports in which enteric bacteria were mixed directly with soil.

INTRODUCTION

Wilderness area is relatively large but the space within it for disposal of human waste is relatively small. Designated campsites, trails, canoe routes, rock climbing routes, rivers and various other physical terrain factors concentrate human users of Wilderness within the area and promote the disposal of feces within relatively small boundaries. For much of the area officially designated as Wilderness, proper construction and maintenance of sanitary waste disposal facilities is impractical (7). Feces are disposed of by being buried, at various depths but shallowly in general, by being placed under rocks or by being left upon the surface. Shallow burial is frequently recommended and some campers carry small trowels for that purpose. An untested assumption of this practice is that intestinal bacteria will die fairly rapidly in shallowly buried feces. This assumption is based upon numerous studies of bacteria directly mixed with soil and upon the knowledge that intestinal microorganisms do not compete effectively with the autochtonous soil microbiota.

Shallow burial of feces does not constitute thorough mixing with soil, however. The system is one of feces placed in soil rather than of feces mixed with soil. With ever increasing use of Wilderness, the question arises as to the longevity of intestinal human pathogens and the possibility of contamination. This question was addressed in a general way by a previous study (13). In the project reported here, the survival of two intestinal (enteric) bacteria was followed in a system closely resembling actual field disposal of feces by shallow burial. This is merely one additional problem in the administration of Wilderness (8), but one which is not adequately understood.

GENERAL PLAN OF STUDY

Two enteric bacteria were chosen which are similar to those normally occurring in feces but which could be readily distinguished and counted. A normal human feces sample was inoculated with these bacteria, buried in soil in a manner closely simulating actual practice by hikers, and specimens retrieved at weekly intervals to count the bacteria.

Six field sites for burial of feces were selected at different elevations on one mountain chain providing very different exposures, soils and vegetation. Samples for burial were prepared in the laboratory using soils obtained from these sites. Soil moisture and soil temperature were measured at the sites at the time of sample retrieval.

A preliminary field study in the summer of 1979 showed greater survival of the bacteria than had been anticipated. In the summer of 1980, the study was repeated with greater replication and with burial at two depths.

The two bacteria were counted by the plate count method and their numbers expressed relative to the weight of feces which had been buried. Sample retrieval and the initiation of counting was completed within the space of one day to minimize changes in numbers during transportation and handling.

In a strict sense, conclusions are limited to the two bacteria studied but these are genetically similar to some human pathogens of interest. The study was not designed to be definitive in regard to any specific human pathogen but was intended to answer in general the question of the survival of enteric bacteria in feces buried in soil.

MATERIALS AND METHODS

Bacteria. A strain of Escherichia coli resistant to 100 µg/ml of streptomycin and to 50 µg/ml of nalidixic acid and a culture of Salmonella typhimurium were each grown in tryptic soy broth (Difco Laboratories) for 18 h, centrifuged and resuspended in sterile reagent-grade water (resistance >10 M cm) at a concentration of approximately 10^9 cells/ml. Each bacterial suspension was then diluted so that 1 ml added to 1g of feces gave a count of that bacterium of approximately 10^6/g of feces. Counts were made on Levine eosin-methylene blue agar containing streptomycin and nalidixic acid and on salmonella-shigella agar respectively.

Inoculated fecal suspension. Feces which did not contain either antibiotic-resistant E. coli or S. typhimurium was blended with an equal weight of sterile water for 5 min in a Sorvall blender. A 3g sample was removed as a control. Bacterial suspensions prepared as described above were added at a rate of 0.1g of bacterial suspension/g feces. The numbers of the bacteria in the inoculated feces was subsequently determined by actual count which is more exact than using the number estimated from making dilutions and using a 1 ml inoculum.

Sample holders. Coulter Counter plastic sample holders (Acuvettes) were adapted by piercing the bottom and sides with a hot wire to make four holes approximately 3 mm in diameter. An aluminum strip was welded to the top of the acuvette to assist in locating it after burial.

Sample preparation. Soil was collected from each site. Three grams of the appropriate soil was placed in the bottom of an acuvette, 3 g of fecal suspension added and another 3 g of soil placed on top. All samples were prepared on the same day and were refrigerated a few days before burial. At the time of burial, the acuvettes were filled to the top with the soil. Acuvettes were buried either at 5 cm or at 20 cm, measuring to the top of the acuvette. A few samples intended for 20 cm burial were buried at depths less than that because bedrock was encountered.

Field Sites. Six sites were selected to give a variety of elevations, exposures, and plant communities while being close enough to the laboratory to allow retrieval of the samples and the initiation of bacterial counting in a single day. These sites were in the Bridger Mountains north of Bozeman, MT, close to the road and trail to Fairy Lake and Sacajawea peak. The area is used heavily by hikers after the road becomes open in early or mid-July and usually closes in September because of snow although it sometimes remains open later.

Site 1. Elevation ca 2730 m, western exposure, on a saddle between Sacajawea and Hardscrabble peaks, exposed to frequent strong winds. Vegetation a dense mat usually under 10 cm in height, continuous except where interrupted by rodent burrows. Dominated by Sibbaldia, Carex, Frasera, Orthocarpus, Cerastium and Potentilla. Soil developed from limestone but may have a loess component from the Gallatin Valley soil below. Soil pH 7.32. Limestone rocks and fragments abundant.

Site 2. Same saddle and elevation but on the eastern side. Soil derived from red dolomitic siltstone, steeper pitch than site 1 and extremely dry. Mostly bare soil and rock. The burial site was partly bare soil and partly a mat of Dryas.

Site 3. Elevation ca 2500 m. At timberline in a pure stand of severely stunted Abies lasiocarpa. Although the soil in which the trees grow is largely a loess, samples were buried in an organic duff under a clump of fir. The burial site was well protected from wind and to a considerable extent from rain.

Site 4. Elevation ca 2377 m. An Abies-Picea forest on a so-called "dry limestone" site. Thalictrum, Ribes, Arnica and Senecio were the predominant understory plants. Site 4 soil was a shallow duff on limestone with little

development of a mineral soil. Some samples could not be buried at 20 cm depth because the soil was too shallow. Soil pH 5.12.

Site 5. Elevation ca 2005 m. A steeply pitched meadow with a dense vegetation whose present composition is presumably the result of grazing and the introduction of seeds. Dominated by Phleum, Delphineum, Rudbeckia, Heracleum, Agropyron and Bromus but containing a large number of other grasses and forbs. Soil developed from sandstone, pH 6.65. Becomes very wet during rainy periods but dries rapidly because of the steep slope and acquires a hard, baked consistency in dry periods. Shallow in places.

Site 6. Elevation ca 1902 m. A small alluvial meadow in Abies lasiocarpa forest. Low pitch and remains wet longer than other sites. Heavy black soil, pH 6.63. Dense stand of Phleum, Agrostis, Bromus, Carex, Geranium, Rudbeckia, Achillea, Potentilla, Delphineum, Arvens, Galium, Heracleum, Mertensia, Fragaria, Rumex and other plants.

Soil moisture. Gymsum blocks were buried at the depth of the samples and soil moisture measured at the time of sample retrieval using a soil moisture meter (Soil Test, Inc.) (15).

Soil temperature. A temperature probe was inserted to the depth of the sample at the time of sample retrieval.

Soil pH. pH was determined on 1:2 soil water slurries (w/v) in the laboratory using a sloped meter and combination electrode, allowing time for equilibrium. These values are the ones cited above in description of the sites. Values determined using a 0.01M $CaCl_2$ solution in place of water are included in a table.

Experimental Procedures. 1979 Field Studies. Sites 1,2,3,4, and 5 were used. Eight replicate soil-feces samples were buried at each site on July 16.

One sample was retrieved from each site each subsequent week with the last sample being taken on Sept. 10. Each sample was emptied into a Waring blendor containing 300 ml water and mixed for 5 min. This slurry was then diluted appropriately for counting the bacteria, based on preliminary experiments. Duplicate plates were prepared using 0.1 ml of each dilution. Bacterial numbers after growth were recorded as the numbers of bacteria/g of starting weight of feces. The preparation of these plates was completed in the evening of the same day that the samples were retrieved, to minimize population changes during handling.

1980 Field Studies. Sites 1, 4, 5, and 6 were used. The same general procedure was followed but with greater replication, and two burial depths were employed. 48 samples were buried at each site, 24 at 5 cm and 24 at 20 cm. A soil corer was used to assist in burial at 20 cm. One gypsum soil moisture block was buried at 20 cm and two blocks at 5 cm, at each site. Three samples were removed from each depth at each site on each succeeding week for five weeks, no samples were removed on the sixth week, but samples were again taken on the seventh and eighth weeks. This left one set of samples in place to be retrieved and counted in the summer of 1981. This will be after the close of this project, but in view of the high survival rates observed, it was thought useful to obtain overwintering data. Samples were buried on July 23 and the last retrieval dates were Sept. 16 and Sept. 17. Samples could not be retrieved from site 1 on Sept. 16 due to strong wind and snow but were obtained the next day when the weather had abated. On all other occasions, all samples were retrieved and counting begun on the same day each week.

RESULTS AND DISCUSSION

1979 Data. Table 1 presents the numbers of the two test bacteria found during the 1979 field study, as the log of the number of cells/g feces. Each figure is the average of two determinations, from one field sample. The same data were used to prepare Fig. 1, in which the range is shown for all five sites for each bacterium each week, and the count at each site is indicated by the location of the number of that site. The principal result from the 1979 field work was that Escherichia coli persisted at $10^{3.5}$ to $10^{4.95}$ cells/g feces at eight weeks and Salmonella typhimurium persisted at levels of $10^{3.94}$ to $10^{4.95}$ cells/g feces for the same time. Site differences were suggestive but not statistically demonstrable. Thus Site 5 had the highest or close to the highest counts for ten of the eighteen readings, while Site 1 was often near the bottom. But the lack of replicate field samples in the 1979 study made conclusions about sites impossible. However, a paired t test comparing the two bacteria based on the combined data from all samplings showed a statistically significant greater survival of S. typhimurium. Table 2 gives the soil moisture readings both in minus bars of water and as actual meter readings. The latter are included because the conversion of meter readings to bars loses some of the information due to the fact that the scales are not linear. There were real differences in soil moisture among sites, especially during the first few weeks, but this was not reflected in the bacterial counts. The water present in the fecal slurry may have nullified soil moisture differences at first but fecal material should come to moisture equilibrium with the soil surrounding it.

The 1979 data have been published (14).

Table 1. Survival of *E. coli* (EC) and *S. typhimurium* (ST) at five field sites, log 10 of cell numbers versus time - 1979 Data.

	Site 1		Site 2		Site 3		Site 4		Site 5	
Week	EC	ST	EC	ST	EC	ST	EC	ST	EC	ST
0	6.91	5.99	6.91	5.99	6.91	5.99	6.91	5.99	6.91	5.99
1	7.08	6.26	8.10	7.14	7.04	6.10	7.32	6.59	8.16	7.34
2	7.27	6.53	7.10	7.25	6.97	7.59	7.27	8.04	7.11	8.00
3	5.26	6.39	6.74	6.50	6.92	6.60	6.50	7.55	7.90	7.89
4	4.63	5.51	5.70	5.56	5.92	5.80	5.53	6.60	5.59	7.61
5	4.60	5.53	5.79	5.59	5.92	5.78	5.06	6.60	6.85	6.71
6	4.23	4.54	4.59	5.25	4.92	4.92	4.70	6.34	4.53	6.76
7	3.95	4.55	4.51	5.20	4.84	4.91	4.52	5.26	3.92	5.28
8	3.87	4.84	4.37	4.95	4.49	4.65	3.86	4.41	3.51	3.94

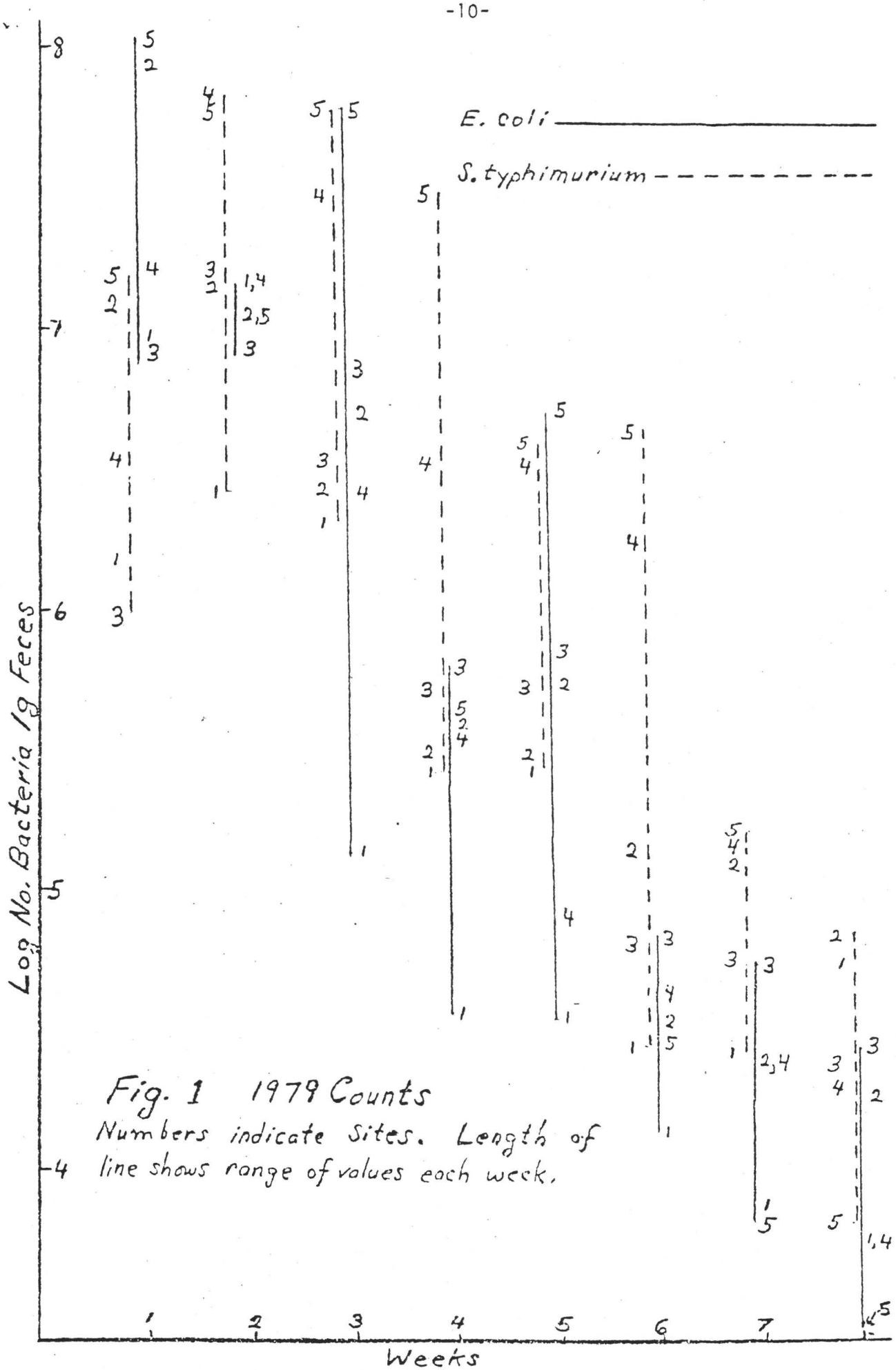


Fig. 1 1979 Counts
Numbers indicate sites. Length of line shows range of values each week.

Table 2. Soil moisture at five field sites in actual meter readings and in minus bars[a] - 1979 data

Week	Site 1		Site 2		Site 3		Site 4		Site 5	
	Meter reading	-bars	Meter reading	-bars	Meter reading	-bars	Meter reading	-bars	Meter reading	-bars
0	178	<1	45	6	72	4	43	6	1	>15
1	187	<1	26	9	55	5	20	>15	153	<1
2	186	<1	108	2	0	>15	18	>15	150	<1
3	151	<1	0	>15	0	>15	5	>15	0	>15
4	180	<1	130	<1	0	>15	198	<1	156	<1
5	186	<1	180	<1	183	<1	185	<1	186	<1
6	187	<1	183	<1	185	<1	187	<1	185	<1
7	186	<1	185	<1	183	<1	185	<1	186	<1
8	162	<1	131	<1	180	<1	188	<1	132	<1

[a] One bar equals 10^5 Pa

1980 Data. Counts were shown graphically for each depth in Figures 2 and 3. The principal conclusion is that survival at eight weeks was on the order of 10^5 to 10^6 cells of S. typhimurium/g feces and 10^6 to 10^7 for E. coli. Beyond this, the survival curves at 5 cm and 20 cm were practically identical. E. coli survived in greater numbers than S. typhimurium in the summer of 1980 but the starting numbers were higher and the curves actually show a flatter slope for S. typhimurium than for E. coli, in agreement with the 1979 data. In the 1980 study, site differences were clearly apparent for E. coli, with Site 6 having the greatest numbers of bacteria for the first five weeks, Sites 1 and 5 being very much alike and Site 4 having the lowest numbers. These differences vanished as the numbers began to drop more rapidly after five weeks. The reasons for the site differences were not apparent and this appears to be an unimportant point. With the close similarity of S. typhimurium at all sites and the general similarity of E. coli at all sites, it is plain that a sanitary problem would be the same at all sites.

Soil temperatures are shown in Fig. 4. In soils, both temperature rise and temperature drop are more extreme and rapid at the surface. Thus, Site 6 showed a large difference in temperature with depth at the August 26 sampling, while the sharp cooling between Sept. 9 and Sept. 16 at Site 1 was more pronounced at 5 cm than at 20 cm. The temperature patterns in Fig. 4 do not bear any consistent relation to the survival curves. E.g. Site 4 was consistently the coolest, but this made no difference in S. typhimurium survival and had no regular correspondence with E. coli survival at other sites. Site 1 had the greatest fluctuation in temperature but this seems to have had little influence on survival. This temperature record is fragmentary, since readings were made only at the actual time of sample retrieval; the time of day varied by a few hours from one site to another and variations during the week were not recorded.

Fig. 2 1950 Counts at 5 cm depth for 8 weeks.
Vertical bars show range of three replicate field samples.
Numbers indicate Sites.

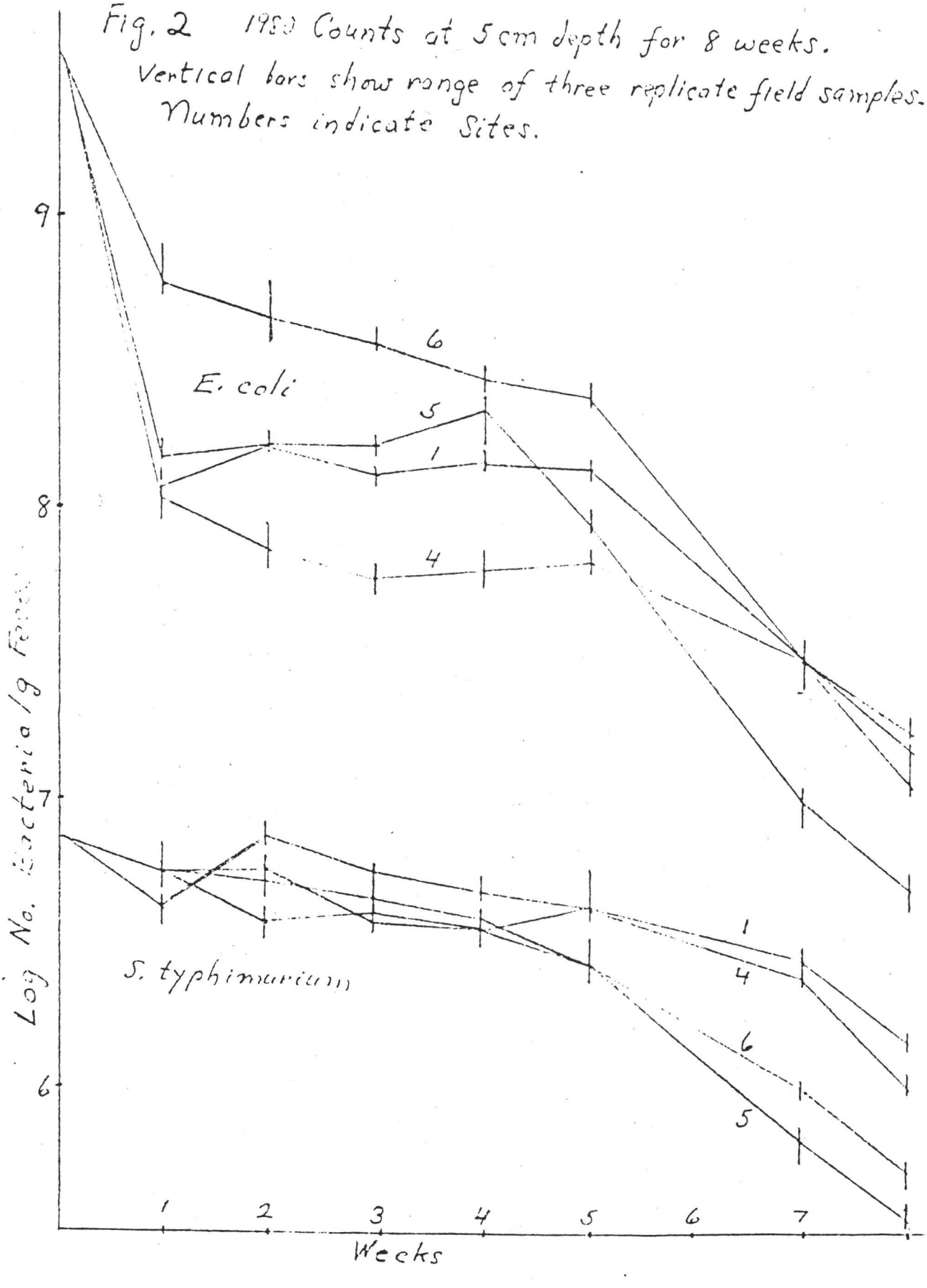


Fig. 3 1980 Counts at 20 cm depth for 8 weeks.
Vertical bars show range of three replicate field samples.
Numbers indicate Sites.

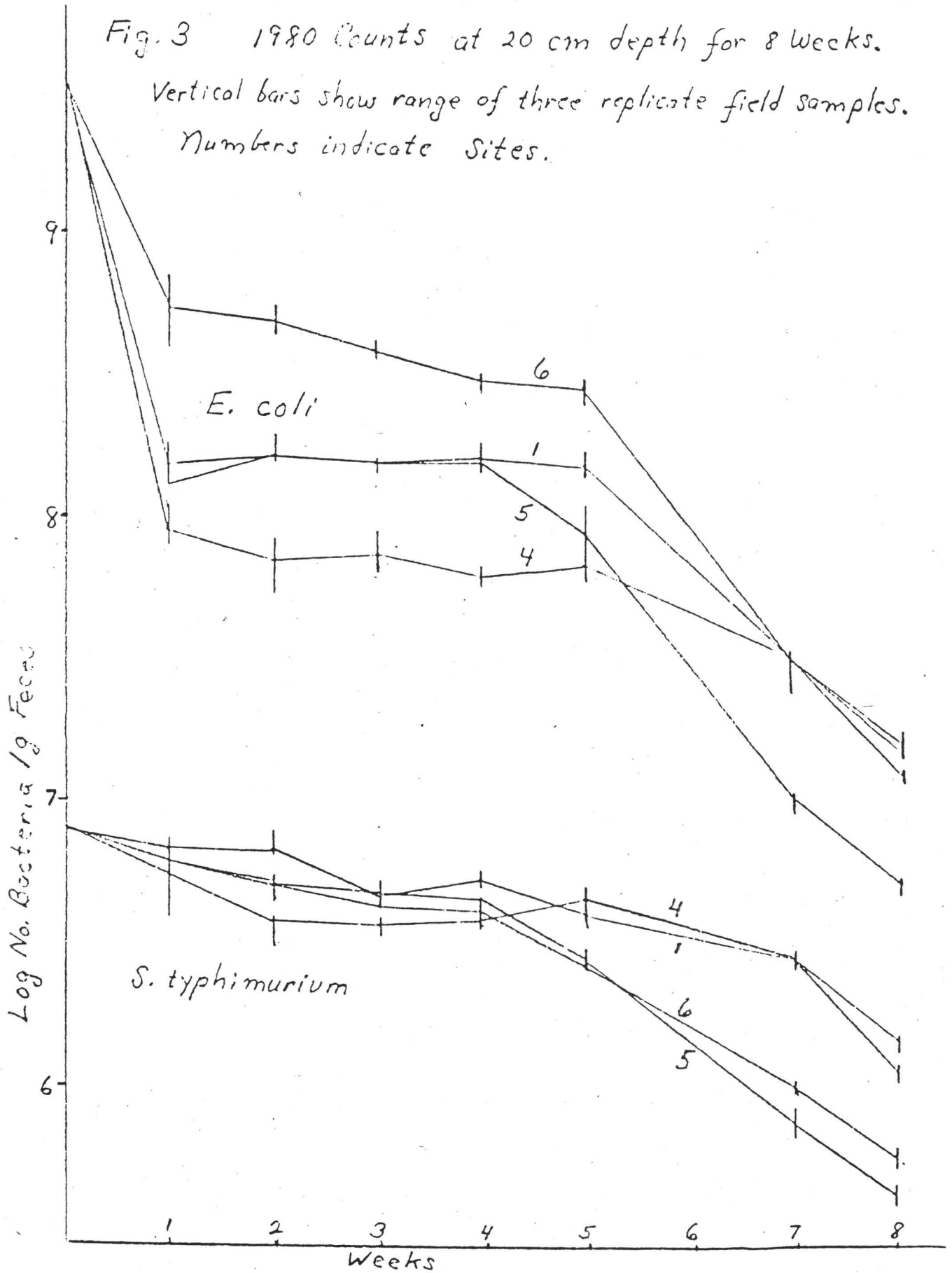
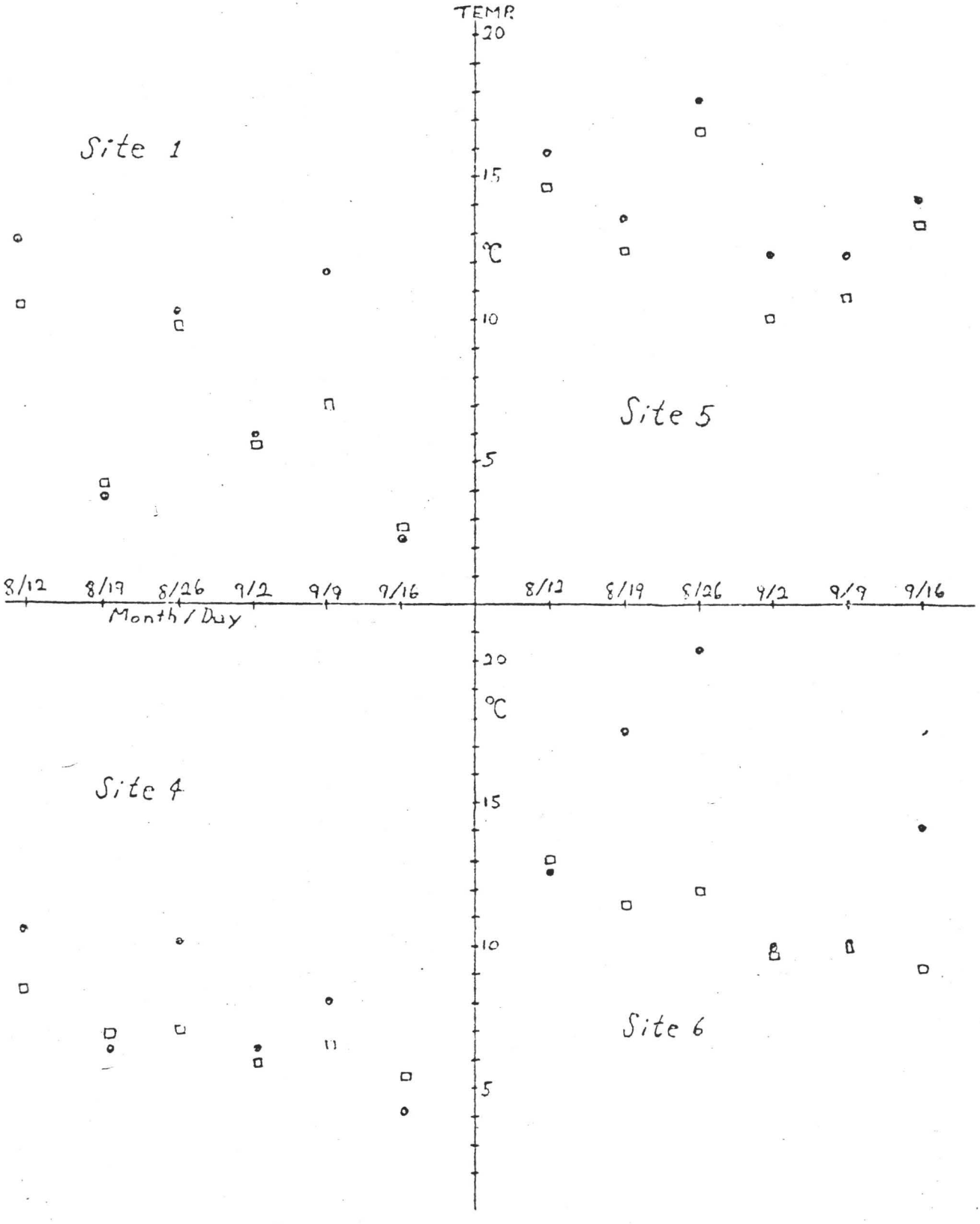


Fig. 4

Soil Temperature 1989 ○ 5 cm □ 20 cm Sites 1,4,5,6



Soil pH was as shown in Table 3.

Table 3. Soil pH. 1:2 mixture of soil and solution (w/v).

Solution	Site 1	4	5	6
Dist. H_2O	7.32	5.12	6.65	6.63
0.01M $CaCl_2$	7.15	4.80	5.36	5.74

The soil pH was apparently not an important factor in survival of bacteria. It must be remembered that the pH of the bacterial environment was the pH of the feces, not the soil. However, it had been expected that soil microbiology related to pH would have some effect on survival and this was not the case.

Soil moisture in 1980 was recorded with a different meter than in 1979 and one which has a scale adapted to drier conditions than we encountered that summer. For this reason, moistures equivalent to a stress condition for plants, for which this meter is designed, were not encountered and no quantitative data are available. All sites were driest at the beginning of the summer when the buried samples still contained water from their preparation and wettest at the latter part of the summer after August 19. Even Site 5 which frequently bakes hard during the summer, did not reach that condition during the 1980 experiment, but was moist to the touch. Bacteria are less subject to water stress than are higher plants, so it can be assumed that water stress was not a factor in the 1980 study. Showers were frequent in the Bridger range during the experiments, although this was not the general weather pattern in 1980.

INTERPRETATION OF RESULTS

This study was designed to test survival of enteric bacteria in feces buried in soil, not the survival of bacteria in soil. The close similarity of results from the top of a mountain ridge, from mature forest, from a high meadow and from a damp meadow at the foot of the mountain, demonstrates conclusively that bacterial survival is primarily a function of the fecal sample rather than of the soil in which it is buried. When it is desired to maintain bacteria for some time, as in laboratory stock cultures, they are dried in the presence of various types of organic matter. Blood serum and skim milk work well, so does sterile soil (11). Feces has not been tested for this purpose but might have the same protective effect. This is a very different situation from active decomposition of feces in aerobic or anaerobic sewage digesters. We know that enteric bacteria die off rapidly in actively decomposing feces and that they do so likewise in soil which is microbially active (1,2,3,4,5,6,9,10,11,12). The actual reasons for their death are obscure but presumably have to do with competition, predation and with chemical changes which damage the cells. For whatever reasons, these factors did not operate rapidly in our experimental design. The potential problem is therefore worse than we anticipated. In particular, the assumption that shallow burial of feces results in a quick die-off of enteric bacteria is wrong.

What can we conclude about the situation where a fecal sample contains virulent human pathogens? Neither E. coli nor S. typhimurium is a virulent human pathogen excent under exceptional circumstances. The most dangerous of the salmonellas, the one causing typhoid fever, reportedly has a shorter life in soil than S. typhimurium and most others of its genus (11), but other reports vary (9,2,3,10). As a working generalization, we can say that most

dangerous pathogens are more highly selected for the environment of the host and consequently less well adapted to life outside the host than are other bacteria. This is not a universal rule, however. Field experiments of the type used in this study are not advisable with active human pathogens. Laboratory studies of the same design that we used in the field, with inoculated feces buried in soil, would be entirely practical. Since we found hardly any differences among extremely different field sites, the laboratory approach would be perfectly valid. Without the data from such experiments, we must conclude that pathogenic enteric bacteria would survive in feces which are disposed of in Wilderness by shallow burial. The same conclusion might or might not be valid for other types of pathogens which could be present in feces, but would certainly be true for some of them. Whether feces containing such pathogens would be disturbed by someone else and cause an infection would depend on a chain of circumstances but would certainly involve the intensity of human use of the area. As a minimum recommendation, human use should be managed in such a way as to rotate the use of fecal disposal areas. The eight week survival we found would make that difficult in many locations. Perhaps the increasing incidence of giardiasis is a harbinger of problems to come.

Our study addressed only burial as a means of waste disposal. Surface disposal is esthetically disturbing but its sanitary significance has not been examined. This question could be answered by rather simple experiments. It is entirely possible that increased exposure would cause a faster die-off. Disposal under rocks is a common practice at higher elevations. Without any data, the first guess is that survival under rocks would be similar to that in our study.

Thorough mixing of feces and soil, provided the material remains moist and exposed to air, would undoubtedly give the fastest killing of enteric bacteria. The problem in establishing this practice by campers is obvious.

This was a study of survival of bacteria in feces buried in soil and did not take in water contamination. However, we have observed several instances of fecal disposal where natural drainage channels were ignored and water transmission was possible.

The shape of the survival curve beyond eight weeks cannot be deduced from our data. Numbers might continue to fall at the same rate, might decrease even more rapidly or might level off. Salmonella causing typhoid has been known to overwinter and cause infections when washed into a reservoir during spring snow melt. The study could not be continued longer at this location; only an exceptionally open fall enabled sampling from the upper sites as late as Sept. 17 in 1980.

CONCLUSIONS AND RECOMMENDATIONS

1. Enteric bacteria, i.e. Escherichia coli and Salmonella typhimurium, when placed in normal human feces and buried in soils, did not die at the rapid rate expected but retained appreciable numbers for at least eight weeks.

2. Bacterial survival was identical at two burial depths, 5 and 20 cm.

3. Sites varying greatly in elevation, exposure and plant community differed only slightly in bacterial survival.

4. Shallow burial of feces by campers may be recommended on esthetic grounds but cannot be relied upon to diminish potential health hazards, if the fecal specimen is later disturbed.

5. Disposal methods other than simple burial should be investigated to obtain comparative bacterial survival rates.

6. The survival of bacteria possessing greater pathogenic potential than those investigated here could be ascertained by laboratory study. Field results in this study indicate that results from laboratory studies would be applicable to field conditions.

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